

Docket No. RDID0013US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of: ZIELENSKI, Ralf

Application No.: 09/760,205

Group No.: 1623

Filed: January 12, 2001

Examiner: To Be Assigned

For: STABILIZED COENZYME SOLUTIONS AND THEIR USE FOR DETERMINING
DEHYDROGENASES OR SUBSTRATES THEREOF

Assistant Commissioner for Patents
Washington, D.C. 20231

PRELIMINARY AMENDMENT

Sir:

Please enter the following amendments prior to examination of the above-referenced
application:

IN THE CLAIMS:

Please cancel claims 1 to 17, without prejudice, in the originally filed application.

Please enter new claims 18 to 45, clean copies of which are shown on the attached pages
as required by 37 C.F.R. 1.121. Because the previous claims have been canceled, no marked up
copies of the previous claims are enclosed herein.

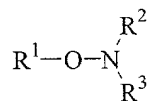
Respectfully submitted,

Date: May 24, 2001

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An aqueous solution comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula

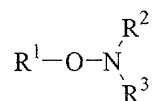


in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group.

19. The solution of claim 18 wherein the organic compound is citric acid or a citrate salt.
20. The solution of claim 19 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
21. The solution of claim 18 wherein the pH is between 1.0 and 7.0.
22. The solution of claim 18 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
23. The solution of claim 22 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
24. The solution of claim 18 further comprising a boric acid derivative.
25. The solution of claim 24 wherein the concentration of the boric acid derivative is about 50 to 200 mM.

26. A method for determining the concentration of a hydrogen-transferring substrate in a sample comprising:

- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring enzyme for the substrate, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula

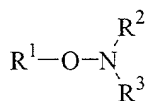


in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, and

- (b) detecting the change in absorbance of the coenzyme as a measure of the concentration of the substrate present in the sample.

27. A method for determining the activity of a hydrogen-transferring enzyme in a sample comprising:

- (a) forming a reaction mixture by combining the sample with a hydrogen-transferring substrate for the enzyme, a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof, and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group, and

(b) detecting the change in absorbance of the coenzyme as a measure of the activity of the enzyme present in the sample.

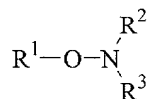
28. The method of claim 26 wherein the analyte is selected from the group consisting of lactate, glutamate, ammonia, alcohol, glyceraldehyde-3-phosphate and glucose.
29. The method of claim 27 wherein the enzyme is selected from the group consisting of dehydrogenases of lactate, glutamate, alcohol, glycerol-3-phosphate and glucose.
30. The method of claim 26 or 27 wherein the organic compound is citric acid or a citrate salt.
31. The method of claim 30 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
32. The method of claim 26 or 27 wherein the pH of the reaction mixture is between about 8.5 and 10.0.
33. The method of claim 26 or 27 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
34. The method of claim 33 wherein the concentration of the hydroxylamine derivative or salt is between about 2 and 300 mM.
35. The method of claim 26 or 27 wherein the reaction mixture further comprises a boric acid derivative.

36. The method of claim 35 wherein the concentration of the boric acid derivative is about 50 to 200 mM.

37. A kit for determining the concentration of a hydrogen-transferring substrate in a sample comprising:

(a) a first reagent comprising a hydrogen-transferring enzyme for the substrate in a buffer having a pH between about 8.5 and 10.0 and

(b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the formula



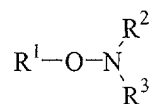
in which R¹, R² and R³ are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group.

38. A kit for determining the activity of a hydrogen-transferring enzyme in a sample comprising:

(a) a first reagent comprising a hydrogen-transferring substrate for the enzyme and a buffer having a pH between about 8.5 and 10.0 and

(b) a second reagent comprising a hydrogen-accepting coenzyme selected from the group consisting of NAD, NADP and derivatives thereof and one or more compounds selected from the group consisting of organic compounds or salts thereof having a pKa value between 1.5 and 6.0 and nitrogen compounds of the

formula



in which R^1 , R^2 and R^3 are the same or different and denote hydrogen or a saturated or unsaturated alkyl or aryl group.

39. The kit of claim 37 or 38 wherein the organic compound is citric acid or a citrate salt.
40. The kit of claim 39 wherein the concentration of the citric acid or citrate salt is about 5 to 200 mM.
41. The kit of claim 37 or 38 wherein the second reagent has a pH between about 1.0 and 7.0.
42. The kit of claim 37 or 38 wherein the nitrogen compound is a hydroxylamine derivative or salt thereof.
43. The kit of claim 42 wherein the concentration of the hydroxylamine derivative or salt is about 2 to 300 mM.
44. The kit of claim 37 or 38 wherein the first reagent further comprises a boric acid derivative.
45. The kit of claim 44 wherein the concentration of the boric acid derivative is about 50 to 200 mM.